content in WZ (4.09±1.13 $\times 10^3$ μg/mL/cm^3) measured by DMMB assay was significantly higher than that in RZ (1.57±0.42 $\times 10^3$ μg/mL/cm3; P = 0.004). There was a significant correlation (R2 = 0.62) between the proteoglycan estimated by sugar area of FT-IRIS and the proteoglycan content measured by DMMB assay.

Conclusions: Collagen was low and proteoglycan was high in WZ, whereas collagen was high and proteoglycan was low in RZ. The results indicate that proportion of collagen and proteoglycan is not uniform but different in WZ and in RZ. Correlations between FT-IRIS analysis and biochemical assays for collagen and for proteoglycan indicate that FT-IRIS may be a useful tool for quantitative analysis of extracellular matrix of meniscus.

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CELLULAR AND BIOMECHANICAL SEGMENTAL CHARACTERIZATION OF HUMAN MENISCUS

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Purpose: The regeneration of meniscus lesions using tissue engineering strategies has recently been attracting great deal of attention. Tissue engineering driven approaches however, require a better knowledge on the segmental composition of meniscus from both biological and biomechanical point of view. With this work we aim to contribute to the knowledge of this tissue aiming future clinical applications in particular, the aspects dealing with the segmental analysis of cellular phenotype and distribution, mechanical properties and extracellular matrix composition.

Methods: Human tissue was obtained from local hospitals by means of surgery or biopsy, in accordance with local ethical committee guidelines. For this study we evaluated menisci from 30 donors, 26 lateral and 23 medial menisci were enrolled for study. Only morphologically intact menisci, not submitted to previous surgery were included. Each meniscus was divided into anterior, middle and posterior segments prior to mechanical, biological or histological evaluation. We isolated human meniscus cells (HMC's) using explants or using an enzymatic standard protocol. Flow cytometry analysis was performed in order to characterize meniscus cells population. Micro-computed tomography (Micro-CT) of freeze-dried meniscus was also carried out. Histomorphometric analysis of menisci stained sections (haematoxylin and eosin – H&E, safaranin O and collagen I) was performed for segmental characterization of ECM and cells distribution. Dynamic mechanical analysis (DMA) was carried out for anterior, middle and posterior segments of fresh menisci. Within the region of interest, samples were cut in cylindrical shapes with 4 mm diameter and 4 mm thickness using a biopsy punch and were stored in PBS solution. The viscoelastic measurements were performed, at 37°C in PBS (pH 7.4), using a TRITEC8000B DMA from Triton Technology (UK), equipped with the compressive mode.

Results: Micro-CT analysis revealed that meniscus (freeze-dried) possessed a mean porosity of 53%, a mean pore size and trabeculae thickness of 85 μm and 80 μm, respectively. DMA analysis has shown, as expected, variability within samples due to their human nature, however we could observe a trend of increasing menisci stiffness: medial anterior (0.25 MPa at 1 Hz) < lateral anterior < lateral middle < medial posterior < lateral posterior < medial middle (0.9 MPa at 1 Hz). Cells isolated from the different samples are a mixed population of cells, i.e. fibrochondrocyte-like and MSCs (cells are positive for CD105, CD73 and CD90, and lack CD34 and CD45). Figure 1 illustrates the histological evaluation and it shows that meniscus ECM is composed of collagen-type I. Moreover, this fibrocartilageneous tissue has higher cell density in the periphery as compared to meniscus core. Cellular density among the different segments (anterior, middle, posterior) of meniscus was quantified using the H&E 2-D histological images.

Conclusions: To our knowledge, this is the first study of segmental characterization of fresh human menisci, without changes due to freezing or cryopreservation, in respect to biomechanical properties, further considering cells phenotype and distribution. This study provides deeper insights on human meniscus properties, contributing for the development of adequate acellular and cellular tissue engineering strategies for the regeneration of meniscus.

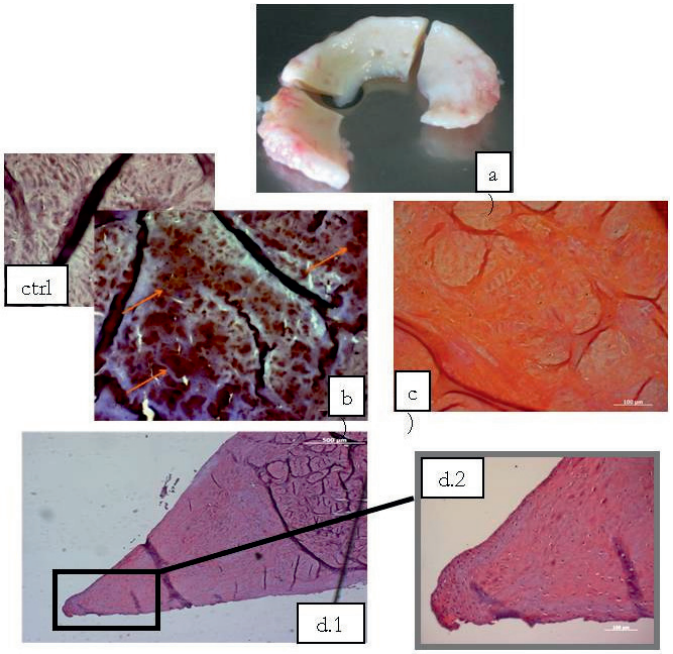

Fig. 1. (a) Menisci segments considered in the different evaluation (anterior, middle and posterior). Evaluation of the (b) presence of collagen type I in the ECM; (c) safranin O staining demonstrating the presence of fibrocartilage distribution; (d) H&E stained sections at different magnification [low (d.1) and high (d.2) magnification].

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EXPERIMENTAL KNEE JOINT PAIN DURING STRENGTH TRAINING INCREASES MUSCLE STRENGTH GAIN IN HEALTHY SUBJECTS: A RANDOMISED CONTROLLED TRIAL

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Purpose: Knee joint pain and reduced quadriceps strength are cardinal symptoms in many knee pathologies such as knee osteoarthritis. In people with painful knee pathologies quadriceps exercise reduce pain, improves physical function and increases muscle strength. A general assumption is that pain compromises muscle function and thus may prevent effective rehabilitation. The aim of the study was to evaluate the effects of experimental knee joint pain during quadriceps strength training on muscle strength gain in healthy individuals.

Methods: Twenty-seven healthy, untrained volunteers participated in a randomized controlled trial of unilateral quadriceps strengthening (8 weeks/3 times per week). Participants were randomized to perform the resistance training during pain induced by injections of either painful hypertonic saline (pain group, N = 13) or a control condition with injection of non-painful isotonic saline (control group, N = 14) into the infra-patellar fat pad. The resistance training consisted of two quadriceps strengthening exercises (leg press and knee extension machine exercises). All participants performed 3 sets of each exercise with loads corresponding to 80% of 1 repetition maximum (RM). Each set was performed to the point of muscular fatigue (inability to maintain the target load; approx. 8–12 repetitions). The primary outcome measure was change in maximal isokinetic muscle strength in knee extension/flexion (60, 120 and 180 deg/s.).

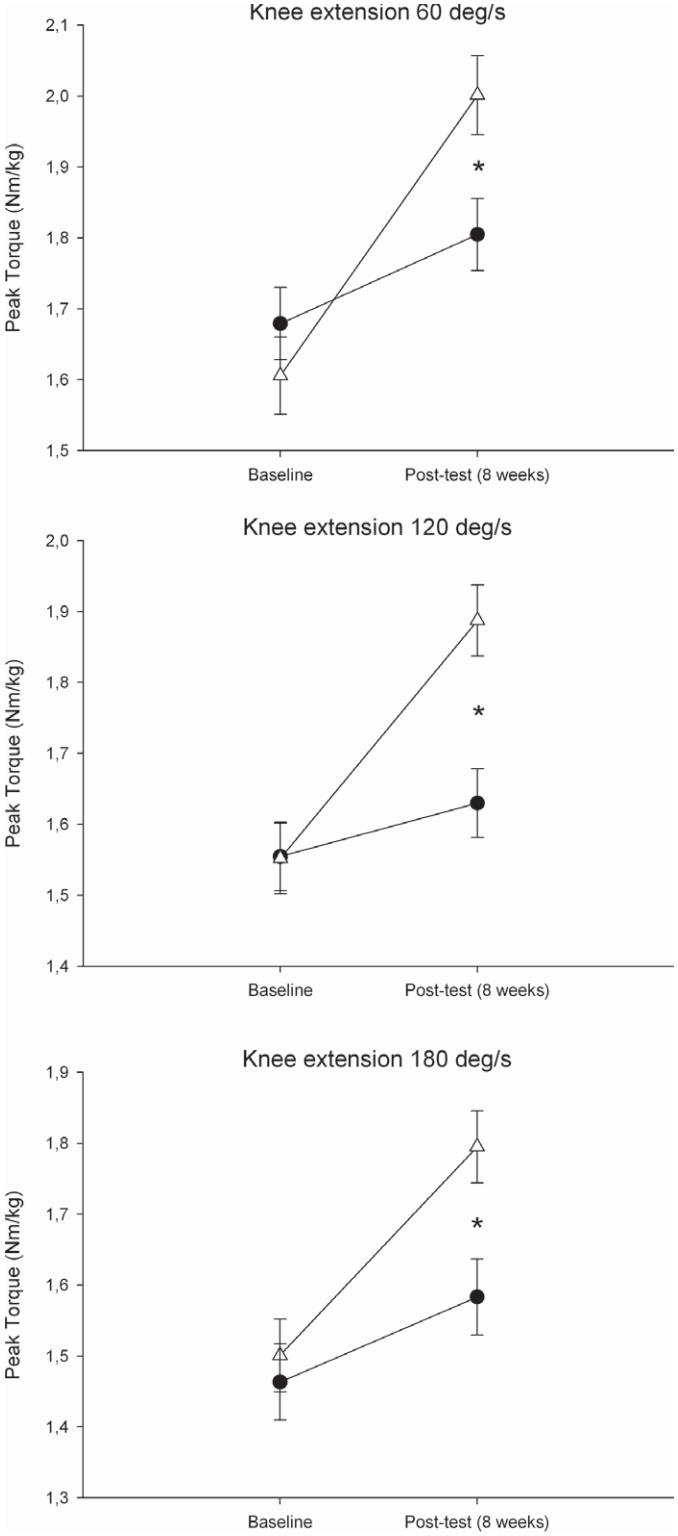

Fig. 1. Peak torque (Nm/kg) in knee extension at baseline and post-test after 8-weeks of exercise intervention for the pain group (triangles) and control group (circles). Measurements were done at 60 deg/s (top), 120 deg/s (middle), and 180 deg/s (bottom). There were statistically significant differences between the groups at all angular velocities at the post-test.

Results: On average the pain group, increased their maximal isokinetic muscle strength in knee extension by 21.9% and the control group by 6.9% (Figure 1). The change in muscle strength was significantly different between groups ($P < 0.0001$) and the improvements were independent of angular velocities ($P = 0.42$). In knee flexion there were improvements in isokinetic muscle strength in both groups ($P < 0.0001$) but with no group differences ($P = 0.80$). Strength improvements were independent of angular velocities ($P = 0.24$).

Conclusion: Experimental knee joint pain improved the training-induced gain in muscle strength following 8-weeks of quadriceps training. It remains to be studied, whether knee joint pain has a positive effect on strength gain in patients with knee pathology.

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RELATIONSHIP BETWEEN ASPECTS OF THE PAIN EXPERIENCE IN KNEE OSTEOARTHRITIS AND FUNCTION AND DISABILITY

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Purpose: In recognition that pain is the most common symptom of knee osteoarthritis (OA), many epidemiologic and therapeutic studies have evaluated knee pain frequency and/or severity. However, the experience of pain has not been well characterized (Hawker 2009). It is widely accepted that knee pain influences physical functioning in knee OA, but how specific aspects of the pain experience relate to function impairment and disability is unclear. Our goal was to analyze separate aspects of the pain experience to determine which aspect is most closely associated with measures of function and disability in persons with knee OA.

Methods: We studied persons with knee OA defined by osteophyte presence in at least one knee. Four aspects of the pain experience were evaluated: pain intensity reported on a 0–10 numeric rating scale (each knee separately) past 30 days; how much has pain affected sleep (ICOAP item); pain after completing 20 m walk (0–10 rating scale); pain catastrophizing (Pain Catastrophizing Scale). Function was evaluated using: the WOMAC function scale; the Late Life Function Instrument (LL-FI), basic and advanced lower extremity function scales; 20 m walk time; time to complete 5 chair stands. Disability was evaluated using: the Late Life Disability Instrument (LL-DI), activity frequency and activity limitation scales. Lower LL-FI and LL-DI scores are worse; higher WOMAC function, 20 m walk time, and chair stand time are worse. We used linear regression with function or disability as the dependent variable, including each pain measure and age, gender, and BMI in each model, after screening for problematic multicollinearity. We calculated standardized regression coefficients, i.e. the estimate of expected change in standard deviation (SD) units in the average value of the dependent variable per SD change in the predictor, after considering all other variables in the model, to compare the strength of association of the different predictors with the outcome variable within the same model.

Table: Standardized coefficients from multipredictor regression models

	WOMAC function	LL-FI, basic LE function	LL-FI, advanced LE function	20 m walk time	Chair stand time	LL-DI, activity frequency	LL-DI, activity limitation
Pain intensity	**0.10**	−0.05	−0.05	0.11	0.06	−0.05	−0.05
Pain affecting sleep	**0.36**	**−0.19**	**−0.14**	0.12	**0.18**	−0.05	−0.12
Pain after 20 m walk	**0.26**	**−0.20**	**−0.29**	0.11	−0.06	−0.06	−0.13
Pain catastrophizing	**0.25**	**−0.29**	**−0.18**	0.11	**0.22**	**−0.23**	**−0.30**
Age	0.04	**−0.17**	**−0.32**	**0.35**	**0.17**	0.05	0.11
BMI	**0.19**	**−0.21**	**−0.30**	**0.36**	0.03	−0.04	−0.08

Results: The sample was comprised of 250 participants [mean age 64.8 (SD 10.2), BMI 28.6 (5.6), 189 (76%) women]. The table shows the standardized coefficients for each model (bold and italicized when significant). The row variables and gender were included in each model. Pain intensity was associated with WOMAC function but no other measure, pain affecting sleep with almost all function measures but no disability measure, and pain after 20 m walk with the self-report function measures. Pain catastrophizing was the only aspect to be associated with the disability measures.

Conclusions: In conclusion, when these four aspects of the pain experience were considered concurrently, pain catastrophizing was most